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9-Oxime-3-ketolides: Modification at the C-11,12-diol moiety and antibacterial activities against key respiratory pathogens

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Abstract—In the search for new types of ketolide antibiotics active against key respiratory pathogens including erythromycin-resistant strains, we conducted an extensive study on the modification at the C-11,12-diol moiety of 9-oxime-3-ketolide derivatives. Among the derivatives prepared, compound **6** with carbonate at the C-11,12 position was found to have potent antibacterial activities against erythromycin-resistant *Staphylococcus aureus* as well as other erythromycin-susceptible strains.

1. Introduction

Macrolide antibiotics such as erythromycin A (EM) have been widely used for the treatment of gram-positive bacterial infection. Some semi-synthetic analogues of erythromycin, such as clarithromycin (CAM) and azithromycin, have also been developed to improve the antibacterial activity or pharmacokinetic profile. However, the extensive clinical application of macrolide antibiotics has resulted in an increasing emergence of macrolide resistance.[1] This situation has led researchers to search for new types of macrolide antibiotics that can overcome this resistance. In recent years, 3-keto derivatives of erythromycin A, the ketolides, were found to be active against macrolide-resistant pathogens, and telithromycin[2] was marketed in 2001 as the first ketolide[3] in the EU. A number of ketolides with the 9-keto functionality,[4] including telithromycin, have been reported, but only a few ketolides with the 9-oxime group[5] are known, and no systematic study on the structure–activity relationship for 9-oxime ketolides has been reported yet. We report herein the preparation of 9-oxime ketolides with various C-11,12-diol moieties and describe their antibacterial activities (see Fig. 1).

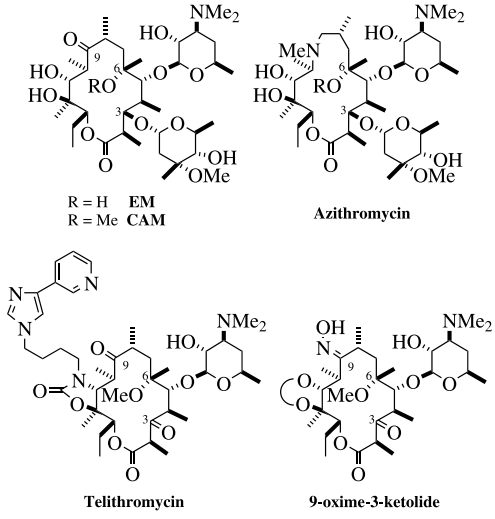

Figure 1. Structure of macrolide antibiotics.

2. Chemistry

Scheme 1 shows the synthesis of compound **6** with C-11,12-carbonate. Treatment of **1**,[6] prepared from erythromycin A, with concentrated HCl in THF gave the deglycosylated compound **2**, which was subsequently subjected to DMSO oxidation and afforded ketolide **3**. The formation of C-11,12-carbonate was accomplished using triphosgene and pyridine in THF giving **4**, which was subsequently deprotected and N-methylated to give **6** bearing C-11,12-carbonate-9-oxime. A compound with cyclic sulfate **11** was prepared as shown in

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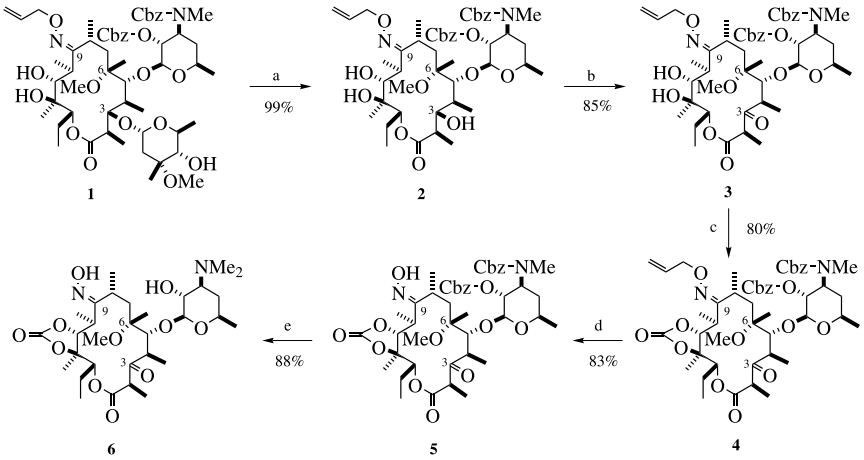
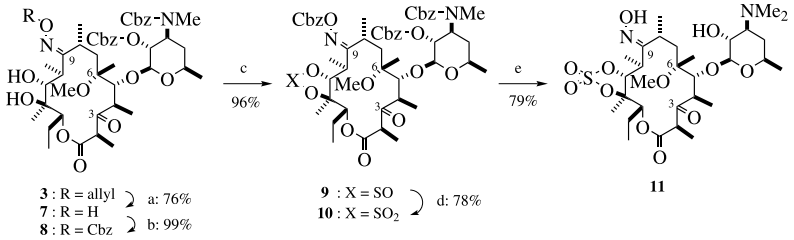


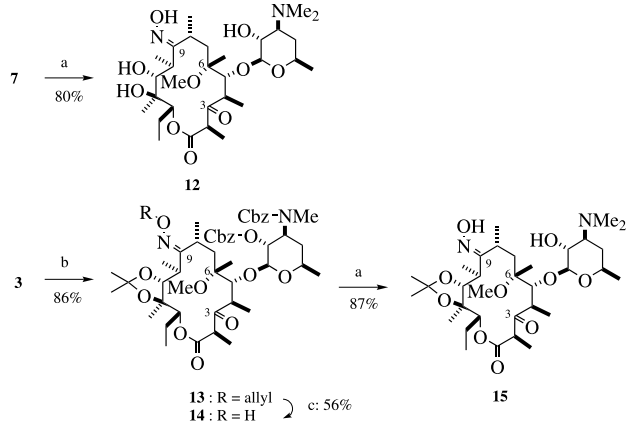
Scheme 1. Reagents: (a) conc. HCl, THF; (b) $(CF_3CO)_2O$, DMSO, NEt_3, CH_2Cl_2; (c) $(CCl_3O)_2CO$, pyridine, THF; (d) $Pd(OAc)_2$, PPh_3, HCO_2H, NEt_3; (e) (1) H_2, 20% $Pd(OH)_2/C$, (2) H_2, 20% $Pd(OH)_2/C$, HCHOaq, acetate buffer (pH 4.4).



Scheme 2. Reagents: (a) $Pd(OAc)_2$, PPh_3, HCO_2H, NEt_3, EtOH–H_2O; (b) CbzCl, $NaHCO_3$, 1,4-dioxane; (c) $SOCl_2$, pyridine, THF; (d) cat. $RuCl_3$, $NaIO_4$, CCl_4–CH_3CN–H_2O; (e) (1) H_2, 20% $Pd(OH)_2/C$, (2) H_2, 20% $Pd(OH)_2/C$, HCHOaq, acetate buffer (pH 4.4).

Scheme 2. Tri carbobenzoxy-protected ketolide **8**, prepared from **3**, was converted to C-11,12-sulfite **9** using $SOCl_2$–pyridine. Treatment of **9** (mixture of diastereomers) with a catalytic amount of $RuCl_3$ and $NaIO_4$ gave compound **10**, which was transformed to **11** bearing C-11,12-sulfate.

Compound **12**[5b] with C-11,12-diol and compound **15** with C-11,12-acetonide were prepared from compounds



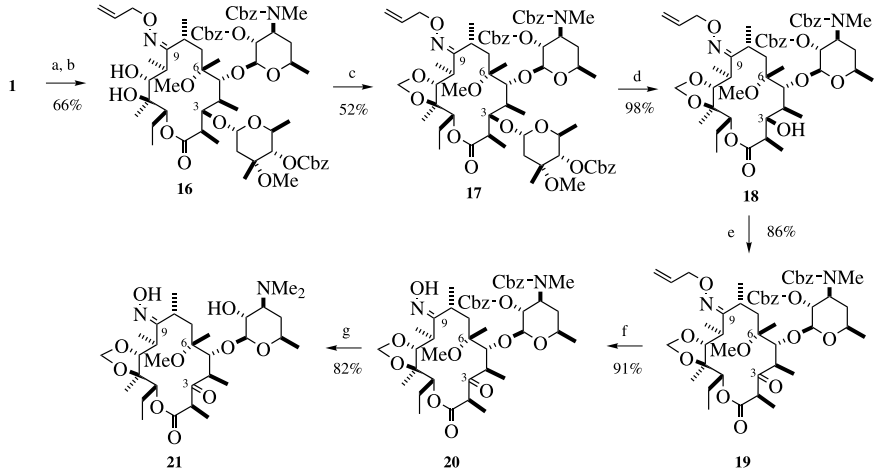
Scheme 3. Reagents: (a) (1) H_2, 20% $Pd(OH)_2/C$, (2) H_2, 20% $Pd(OH)_2/C$, HCHOaq, acetate buffer (pH 4.4); (b) $Me_2C(OMe)_2$, *p*-TsOHEH$_2$O, $(CH_2Cl)_2$, reflux; (c) $Pd(OAc)_2$, PPh_3, HCO_2H, NEt_3, EtOH–H_2O.

7 and **3**, respectively (Scheme 3). Acetonide formation at the C-11,12-diol moiety giving **13** was accomplished using 2,2-dimethoxypropane and *p*-toluenesulfonic acid as an acid catalyst.

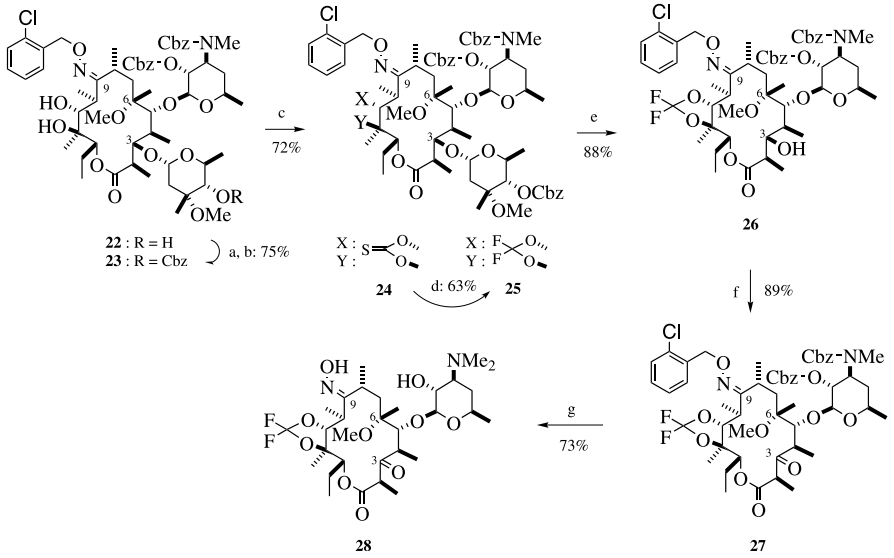
Schemes 4 and 5 show the synthesis of compounds **21** and **28** bearing C-11,12-methyleneacetal and difluoromethyleneacetal moieties. Compounds **3** and **8** could not be used as key intermediates for the synthesis of **21** and **28**, because C-2 alkylation occurred under the conditions of using alkyl halide and strong base. Therefore, we used compounds **16** and **23** bearing C-11,12-diol-3-cladinose as substrates for modification of the C-11,12 moiety. After functionalization of the C-11,12 moiety and subsequent removal of the sugar moiety at C-3, oxidation of C-3 hydroxyl and deprotection/N-methylation resulted in obtaining **21** and **28**, respectively, as illustrated in Schemes 4 and 5.

3. Antibacterial activities

The antibacterial activities (MICs) of **6**, **11**, **12**, **15**, **21**, **28** and CAM[7] as a reference compound against both erythromycin-susceptible and erythromycin-resistant *Staphylococcus aureus* and *Streptococcus pneumoniae*, as well as one strain of *Haemophius influenzae* are shown in Table 1. MICs were determined by the standard serial twofold agar dilution method using Mueller–Hinton agar.

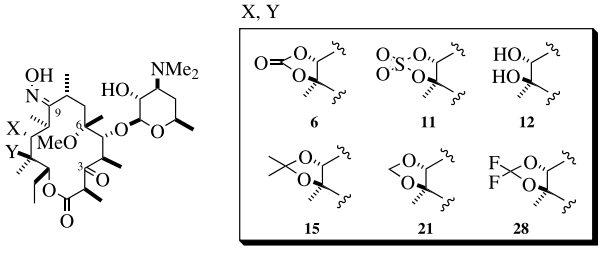


Scheme 4. Reagents: (a) Im_2CO, K_2CO_3; (b) BnOH, NaH, THF; (c) $ClCH_2I$, NaH, DMF; (d) conc. HCl, MeOH; (e) $(CF_3CO)_2O$, DMSO, NEt_3, CH_2Cl_2; (f) $Pd(OAc)_2$, PPh_3, HCO_2H, NEt_3, EtOH–H_2O; (g) (1) H_2, 20% $Pd(OH)_2$/C, (2) H_2, 20% $Pd(OH)_2$/C, HCHOaq, acetate buffer (pH 4.4).



Scheme 5. Reagents: (a) Im_2CO, K_2CO_3; (b) BnOH, NaH, THF; (c) CS_2, MeI, NaH, THF; (d) Et_2NSF_3, CH_2Cl_2; (e) conc. HCl, MeOH; (f) $(CF_3CO)_2O$, DMSO, NEt_3, CH_2Cl_2; (g) (1) H_2, 20% $Pd(OH)_2$/C, (2) H_2, 20% $Pd(OH)_2$/C, HCHOaq, acetate buffer (pH 4.4).

Table 1. In vitro antibacterial activities of **6**, **11**, **12**, **15**, **21**, and **28**



Strain	MIC (μg/mL)						
	6	**11**	**12**	**15**	**21**	**28**	CAM
S. aureus Smith	0.2	>100	0.2	0.78	0.2	0.2	0.2
S. aureus SR17347(EM-R)	0.2	>100	3.13	1.56	0.78	0.39	>100
S. pneumoniae Type I	0.025	100	0.1	0.2	0.05	0.025	0.025
S. pneumoniae SR16651(EM-R)	>100	>100	>100	>100	>100	>100	>100
H. influenzae SR88562 strain	6.25	>100	12.5	>100	25	25	3.13

Comparison of compound **12** and CAM indicates that ketolide **12** is inferior to CAM for activity against both erythromycin-susceptible *S. pneumoniae* and *H. influenzae* though it has potent activity against erythromycin-resistant *S. aureus*. For improvement of activities against both erythromycin-susceptible *S. pneumoniae* and *H. influenzae*, five cyclic C-11,12 derivatives were prepared and their antibacterial activities were evaluated. Among them, compound **6** with C-11,12-carbonate was found to have significantly enhanced activity against erythromycin-resistant *S. aureus* and erythromycin-susceptible *S. pneumoniae*, and to have twofold more potent activity against *H. influenzae* than compound **12**. However, it was disappointing to find that it did not show any activity against erythromycin-resistant *S. pneumoniae*.

To investigate the steric effect of the C-11,12 moiety, two compounds with cyclic acetal **15** and **21** were evaluated. Compared to compound **12**, compound **15** showed less activity against almost all strains except for erythromycin-resistant *S. aureus*. Compound **21** showed potent activities against almost all strains except for *H. influenzae*. These results suggest that the steric effect on the C-11,12 moiety influences the binding of ketolides with bacterial ribosome.[8] In the case of compound **11** with cyclic sulfate, it was almost inactive against all the strains tested. The great difference of activities between **6** and **11** was also presumably due to the steric factor of the C-11,12 moiety, and the sterically hindered C-11,12 moiety is considered to be unfavorable for binding with bacterial ribosome. Compound **28** bearing C-11,12-difluoromethyleneacetal showed slightly superior activities to compound **21** with C-11,12-methyleneacetal. It is possible that the electronic effect on the C-11,12 moiety does not influence the binding to ribosome as much as the steric effect. With respect to the activity against erythromycin-resistant *S. aureus*, all cyclic C-11,12 moieties except for cyclic sulfate were found to be preferable.

In conclusion, we prepared various cyclic C-11,12-modified-9-oxime-3-ketolides and found that the C-11,12 cyclic carbonate has the best functionality at the C-11,12-diol moiety. Compound **6** bearing the C-11,12-carbonate showed the most potent activities against erythromycin-resistant *S. aureus* (MIC: 0.2 μg/mL) and moderate activity against *H. influenzae* (MIC: 6.25 μg/mL), though it was inactive against erythromycin-resistant *S. pneumoniae*. For the improvement of activity against *H. influenzae* and erythromycin-resistant *S. pneumoniae*, we should study modification of the C-9 oxime moiety, which will be reported elsewhere in the future.

4. Experimental

Infrared (IR) spectra were recorded on a JASCO FT/IR-700 spectrometer. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Varian Gemini-300 spectrometer. Chemical shifts are reported in ppm using tetramethylsilane (TMS) as an internal standard. HR-FAB/MS were recorded on a JEOL LMS-SX/SX 102A. Analytical thin layer chromatography (TLC) was carried out on Merck precoated TLC plates silica gel 60 F_{254} and visualized with UV light or 10% H_2SO_4 containing 5% ammonium molybdate and 0.2% ceric sulfate. Flush chromatography was performed with Merck silica gel 60 (230–400 mesh).

4.1. Measurement of in vitro antibacterial activity

MICs were determined using a serial twofold dilution method in Sensitivity Disk Agar-N (Nissui Pharmaceutical, Tokyo, Japan). The overnight cultures of antibacterial strains in Mueller–Hinton broth (Becton Dickinson) were diluted to about 10^6 CFU/mL. Bacterial suspensions of 1 μL were spotted onto agar plates containing various concentrations of an antibiotic and incubated for 20 h at 37 °C before the MICs were scored.

4.2. Preparation of compound 2

To a solution of **1** (60 g, 56.7 mmol) in THF (250 mL) was added concentrated HCl (12 N, 4.8 mL) under cooling with an ice-water bath, and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was carefully quenched with 5% aqueous $NaHCO_3$ (300 mL), and extracted with AcOEt (200 mL). The aqueous layer was extracted with AcOEt (200 mL) again, and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silicagel (*n*-hexane/AcOEt = 4:1–3:2) to give 50.8 g of **2** as a colorless foam (99%).

^{1}H NMR ($CDCl_3$) δ 2.82, 2.86 (3H, two s), 2.94, 2.96 (3H, two s); ^{13}C NMR ($CDCl_3$) δ 7.30, 10.4, 15.2, 15.3, 16.3, 18.4, 19.2, 20.7, 21.1, 26.2, 28.8, 32.8, 35.7, 35.8, 36.2, 36.9, 44.1, 49.8, 54.8, 67.0, 67.1, 68.2, 69.4, 70.4, 73.8, 74.4, 74.5, 77.8, 77.9, 78.0, 81.0, 99.2, 99.3, 117.3, 127.4, 127.5, 127.7, 128.1, 128.2, 128.3, 134.1, 134.9, 135.1, 136.5, 154.2, 154.3, 155.9, 156.2, 169.5, 174.3; MS (FAB) 899^+ ($M+H^+$); HRMS (FAB) calcd for $C_{48}H_{71}N_2O_{14}$ ($M+H^+$): 899.4905; found 899.4902; IR (KBr) 3503, 3066, 3033, 2976, 2938, 2878, 2831, 1752, 1732, 1706, 1629, 1497, 1455, 1406, 1380, 1334, 1292, 1255, 1165, 1120, 1076, 1002 (cm^{-1}).

4.3. Preparation of compound 3

To a solution of DMSO (16.8 mL) in CH_2Cl_2 (250 mL) was added $(CF_3CO)_2O$ (16.8 mL) at −78 °C, and the mixture was stirred at −78 °C for 20 min. To this solution, 50.8 g (56.5 mmol) of **2** diluted with CH_2Cl_2 (250 mL) was added dropwise at −78 °C and the reaction mixture was stirred at −78 °C for 1.5 h. NEt_3 (41.2 mL) was added dropwise to the reaction mixture at −78 °C and stirred for additional 1 h. The reaction mixture was poured into saturated aqueous NH_4Cl and extracted with $CHCl_3$ (150 mL). The aqueous layer was extracted with $CHCl_3$ (150 mL) again, and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue

was purified by column chromatography on silicagel (*n*-hexane/AcOEt = 8:1–2:1) to give 42.8 g of compound **3** as a colorless foam (85%).

^{1}H NMR ($CDCl_3$) δ 2.71, 2.73 (3H, two s), 2.81, 2.85 (3H, two s); ^{13}C NMR ($CDCl_3$) δ 13.7, 13.9, 14.0, 15.1, 16.3, 18.4, 19.4, 20.6, 21.5, 26.1, 28.9, 32.8, 35.7, 36.2, 37.4, 45.6, 45.7, 49.5, 50.7, 54.9, 67.0, 67.1, 68.5, 69.3, 69.6, 70.1, 73.5, 74.5, 76.5, 77.5, 78.0, 78.1, 100.1, 117.5, 127.4, 127.5, 127.6, 127.7, 128.1, 128.2, 128.3, 134.0, 135.0, 135.2, 136.5, 154.2, 154.3, 155.9, 156.2, 169.0, 169.1, 205.1, 205.5; MS (FAB) 897^+ ($M+H^+$); HRMS (FAB) calcd for $C_{48}H_{69}N_2O_{14}$ ($M+H^+$): 897.4749; found 897.4742; IR (KBr) 3513, 3417, 3066, 3033, 2977, 2938, 2877, 1748, 1705, 1497, 1455, 1406, 1380, 1333, 1292, 1254, 1169, 1115, 1069 (cm^{-1}).

4.4. Preparation of compound 4

To a solution of compound **3** (480 mg, 0.54 mmol) in THF (6 mL) was added pyridine (210 μL, 2.6 mmol), $(CCl_3O)_2CO$ (208 mg, 0.7 mmol) at 0 °C, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was cooled to 0 °C and then saturated aqueous $NaHCO_3$ was carefully added to the reaction mixture, which was then stirred for 0.5 h at 0 °C. The reaction mixture was extracted with AcOEt (20 mL). The aqueous layer was extracted with AcOEt (20 mL) again and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silicagel (*n*-hexane/AcOEt = 6:1–1:1) to give 400 mg of compound **4** as a colorless foam (80%).

^{1}H NMR ($CDCl_3$) δ 2.65 (3H, s), 2.81, 2.85 (3H, two s); ^{13}C NMR ($CDCl_3$) δ 10.4, 13.1, 13.8, 15.4, 15.6, 15.7, 18.8, 19.6, 20.6, 22.5, 26.0, 28.9, 33.0, 35.7, 36.2, 37.8, 47.1, 47.2, 49.5, 51.0, 54.8, 67.1, 67.2, 68.6, 69.2, 69.4, 74.5, 74.6, 76.4, 76.5, 76.9, 77.3, 78.1, 78.2, 82.8, 84.4, 100.6, 116.9, 127.4, 127.5, 127.7, 128.0, 128.2, 134.3, 135.3, 135.4, 136.5, 154.1, 154.3, 155.9, 156.3, 163.7, 168.7, 203.4, 203.6; MS (FAB) 923^+ ($M+H^+$); HRMS (FAB) calcd for $C_{49}H_{67}N_2O_{15}$ ($M+H^+$): 923.4541; found 923.4545; IR (KBr) 3428, 3066, 3032, 2976, 2938, 2880, 1811, 1752, 1704, 1644, 1497, 1455, 1382, 1330, 1292, 1254, 1167, 1114, 1067 (cm^{-1}).

4.5. Preparation of compound 5

To a solution of compound **4** (490 mg, 0.53 mmol) in EtOH (10 mL) was added THF (10 mL) and PPh_3 (21 mg, 0.08 mmol), $Pd(OAc)_2$ (6 mg, 0.027 mmol), HCO_2H (61 μL, 1.62 mmol), NEt_3 (227 μL, 1.62 mmol) at room temperature, and the mixture was refluxed for 90 min. The reaction mixture was cooled to room temperature and then concentrated under reduced pressure to evaporate EtOH. The residue was dissolved in AcOEt, washed with saturated NH_4Cl, and brine, dried over anhydrous $MgSO_4$, and evaporated at reduced pressure. The residue was purified by column chromatography on silicagel (*n*-hexane/AcOEt = 16:1–1:1) to give 389 mg of compound **5** as a colorless foam (83%).

^{1}H NMR ($CDCl_3$) δ 2.65 (3H, s), 2.81, 2.85 (3H, two s); ^{13}C NMR ($CDCl_3$) δ 10.2, 13.1, 13.8, 15.4, 15.5, 15.6, 18.6, 19.6, 20.6, 22.3, 25.1, 28.7, 32.7, 35.7, 36.1, 37.9, 47.0, 47.1, 49.5, 50.9, 54.6, 67.0, 67.1, 68.6, 69.2, 69.4, 74.7, 76.1, 76.2, 78.1, 78.3, 82.7, 84.2, 100.4, 100.5, 127.3, 127.5, 127.6, 127.7, 128.0, 128.1, 128.2, 128.3, 135.2, 135.3, 136.4, 153.5, 154.1, 154.2, 155.8, 156.2, 164.5, 168.7, 203.2, 203.4; MS (FAB) 883^+ ($M+H^+$); HRMS (FAB) calcd for $C_{46}H_{63}N_2O_{15}$ ($M+H^+$): 883.4229; found 883.4231; IR (KBr) 3422, 3090, 3065, 3032, 2975, 2938, 2880, 1812, 1752, 1703, 1587, 1497, 1455, 1382, 1352, 1330, 1289, 1253, 1167, 1113, 1085, 1067, 1049 (cm^{-1}).

4.6. Preparation of compound 6

Compound **5** (150 mg, 0.17 mmol) was diluted with EtOH (15 mL) and 0.2 M acetate buffer (3 mL, pH 4.4). To this solution was added 20% $Pd(OH)_2$/C 54 mg (0.1 mmol) with stirring at room temperature under H_2 atmosphere for 1.5 h. After confirming the disappearance of compound **5** by TLC, 37% aqueous HCHO (1.3 mL) was added to the reaction mixture, which was stirred at room temperature under H_2 atmosphere for an additional 2.5 h. The mixture was filtered and concentrated. After being diluted with water, the mixture was basified with 5% aqueous $NaHCO_3$ and then extracted with AcOEt. The resultant residue was purified by column chromatography on silicagel ($CHCl_3$/MeOH = 80:1–10:1) to give 94 mg of compound **6** as a colorless foam (88%).

^{1}H NMR ($CDCl_3$) δ 0.89 (3H, t, J = 7.5 Hz), 0.99 (3H, d, J = 6.9 Hz), 1.24 (3H, d, J = 6.0 Hz), 1.25 (3H, d, J = 6.3 Hz), 1.27 (3H, d, J = 7.5 Hz), 1.37 (3H, d, J = 6.9 Hz), 1.48 (3H, s), 1.55 (3H, s), 1.20–1.72 (5H, m), 1.9 (1H, m), 2.27 (6H, s), 2.50 (2H, m), 2.68 (3H, s), 3.04 (1H, quintet, J = 7.8 Hz), 3.20 (1H, dd, J = 7.3 and 9.9 Hz), 3.57 (2H, m), 3.83 (1H, q, J = 6.7 Hz), 4.20 (1H, d, J = 8.2 Hz), 4.30 (1H, d, J = 7.3 Hz), 4.80 (1H, s), 5.04 (1H, dd, J = 2.5 and 10.1 Hz); ^{13}C NMR ($CDCl_3$) δ 10.2, 13.0, 14.2, 15.4, 15.8, 18.6, 19.7, 21.1, 22.2, 25.3, 28.3, 32.6, 38.3, 40.2, 47.8, 49.7, 51.0, 65.7, 69.3, 70.4, 76.1, 78.4, 79.4, 82.9, 84.4, 103.7, 153.9, 164.7, 169.1, 203.7; MS (FAB) 629^+ ($M+H^+$); HRMS (FAB) calcd for $C_{31}H_{53}N_2O_{11}$ ($M+H^+$): 629.3649; found 629.3646; IR (KBr) 3433, 2974, 2938, 2879, 2787, 1810, 1752, 1717, 1636, 1456, 1381, 1322, 1305, 1284, 1252, 1233, 1220, 1167, 1142, 1110, 1083, 1048, 1006 (cm^{-1}).

4.7. Preparation of compound 7

To a solution of compound **3** (4.5 g, 5 mmol) in EtOH (90 mL) was added PPh_3 (198 mg, 0.75 mmol), $Pd(OAc)_2$ (56 mg, 0.25 mmol), HCO_2H (549 μL, 14.5 mmol), NEt_3 (2.1 ml, 15 mmol) at room temperature, and the mixture was refluxed for 60 min. The reaction mixture was cooled to room temperature and then concentrated under reduced pressure to evaporate EtOH. The residue was dissolved in AcOEt, washed with saturated aqueous NH_4Cl, and brine, dried over anhydrous $MgSO_4$, and evaporated at reduced pressure. The residue was purified by column chromatography on

silicagel (*n*-hexane/AcOEt = 8:1–1:1) to give 3.26 g of compound **7** as a colorless foam (76%).

^{1}H NMR ($CDCl_3$) δ 2.72 (3H, s), 2.81, 2.85 (3H, two s); ^{13}C NMR ($CDCl_3$) δ 10.6, 13.9, 14.9, 16.3, 18.4, 19.5, 20.6, 21.5, 25.2, 28.9, 32.8, 35.8, 36.2, 37.5, 45.9, 49.8, 50.8, 54.9, 67.1, 67.2, 68.6, 69.4, 69.6, 70.3, 73.7, 74.6, 76.5, 76.9, 77.4, 77.6, 78.0, 78.1, 100.2, 127.4, 127.5, 127.7, 127.8, 128.2, 128.3, 128.4, 135.1, 135.2, 136.5, 154.2, 154.3, 156.0, 156.3, 169.2, 169.6, 205.0, 205.3; MS (FAB) 857^+ ($M+H^+$); HRMS (FAB) calcd for $C_{45}H_{65}N_2O_{14}$ ($M+H^+$): 857.4436; found 857.4438; IR (KBr) 3421, 3065, 3033, 2976, 2938, 2877, 1748, 1706, 1640, 1587, 1497, 1455, 1406, 1381, 1333, 1292, 1253, 1168, 1114, 1086, 1069, 1034 (cm^{-1}).

4.8. Preparation of compound 8

To a solution of compound **7** (429 mg, 0.5 mmol) in 1,4-dioxane (9 mL) was added $NaHCO_3$ (63 mg, 7.5 mmol), CbzCl (93 μL, 0.65 mmol) at room temperature, and the mixture was stirred at room temperature overnight. To the reaction mixture was added the saturated aqueous NH_4Cl, and extracted with AcOEt (20 mL). The aqueous layer was extracted with AcOEt (20 mL) again, and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silicagel (toluene/AcOEt = 10:1–6:1) to give 490 mg of compound **8** as a colorless foam (99%).

^{1}H NMR ($CDCl_3$) δ 2.48 (3H, s), 2.81, 2.85 (3H, two s); ^{13}C NMR ($CDCl_3$) δ 10.5, 13.1, 14.0, 14.8, 16.5, 18.0, 19.4, 20.6, 21.6, 27.8, 28.9, 34.1, 35.7, 36.2, 37.4, 44.9, 49.2, 50.6, 54.9, 66.8, 67.1, 67.2, 68.5, 69.3, 69.6, 69.8, 69.9, 73.8, 74.5, 75.3, 75.4, 77.4, 78.2, 78.3, 99.8, 127.4, 127.5, 127.6, 127.7, 127.9, 128.0, 128.2, 128.3, 128.4, 128.5, 134.8, 135.0, 135.1, 136.0, 136.5, 153.3, 154.2, 154.3, 155.9, 156.2, 156.3, 169.2, 177.0, 205.4, 205.8; MS (FAB) 991^+ ($M+H^+$); HRMS (FAB) calcd for $C_{53}H_{71}N_2O_{16}$ ($M+H^+$): 991.4804; found 991.4825; IR (KBr) 3443, 3090, 3065, 3034, 2977, 2939, 2878, 1956, 1745, 1704, 1637, 1605, 1497, 1455, 1405, 1381, 1334, 1254, 1232, 1171, 1114, 1083, 1066 (cm^{-1}).

4.9. Preparation of compound 9

To a solution of compound **8** (498 mg, 0.50 mmol) in THF (10 mL) was added $SOCl_2$ (72.3 μL, 1 mmol) and pyridine (121 μL, 1.5 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 30 min. The reaction mixture was poured into saturated aqueous $NaHCO_3$ and extracted with AcOEt (20 mL). The aqueous layer was extracted with AcOEt (20 mL) again, and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silicagel (toluene/AcOEt = 10:1–8:1) to give 500 mg of compound **9** as a colorless foam (96%).

^{1}H NMR ($CDCl_3$) δ 2.64, 2.69 (3H, two s), 2.81, 2.82, 2.85, 2.86 (3H, four s); ^{13}C NMR ($CDCl_3$) δ 10.0, 10.6, 13.8, 14.0, 14.2, 14.3, 14.5, 14.6, 16.5, 18.7, 19.6, 19.7, 20.6, 22.3, 22.8, 27.8, 28.8, 33.5, 35.6, 36.1, 37.7, 38.0, 45.9, 49.5, 49.8, 50.8, 51.2, 54.6, 66.7, 67.1, 67.2, 68.6, 69.1, 69.2, 69.3, 69.4, 69.6, 69.7, 74.4, 74.5, 76.7, 77.5, 77.6, 77.7, 89.4, 90.1, 100.0, 127.3, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 134.8, 134.9, 135.1, 135.2, 135.3, 136.0, 136.4, 153.3, 153.4, 154.0, 154.1, 154.2, 155.8, 156.2, 156.4, 168.6, 168.7, 173.4, 204.1, 204.5; MS (FAB) 1037^+ ($M+H^+$); HRMS (FAB) calcd for $C_{53}H_{69}N_2O_{17}S$ ($M+H^+$) 1037.4317; found 1037.4301; IR (KBr) 3484, 3391, 3090, 3065, 3033, 2976, 2939, 2880, 1752, 1702, 1633, 1604, 1498, 1455, 1383, 1330, 1255, 1163, 1114, 1066, 1029 (cm^{-1}).

4.10. Preparation of compound 10

To a solution of compound **9** (500 mg, 0.48 mmol) in CH_3CN (10 mL) was added CCl_4 (10 mL) and H_2O (10 mL). $RuCl_3{\cdot}nH_2O$ (20 mg, 0.096 mmol) and $NaIO_4$ (412 mg, 1.93 mmol) were added to the mixture at room temperature with stirring for 1 h. This reaction mixture was poured into saturated aqueous $NaHCO_3$ and extracted with AcOEt (40 mL). The aqueous layer was extracted with AcOEt (40 mL) again, and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silicagel (toluene/AcOEt = 8:1–4:1) to give 395 mg of compound **10** as a colorless foam (78%).

^{1}H NMR ($CDCl_3$) δ 2.64, 2.73 (3H, two s), 2.81, 2.85 (3H, two s); ^{13}C NMR ($CDCl_3$) δ 10.0, 13.8, 14.1, 14.7, 16.6, 18.8, 19.6, 20.6, 22.1, 27.9, 28.8, 28.9, 34.2, 35.6, 36.1, 37.9, 46.6, 49.7, 50.9, 54.7, 67.0, 67.1, 68.6, 69.2, 69.4, 69.7, 74.9, 75.5, 78.1, 90.0, 100.4, 127.3, 127.4, 127.6, 127.7, 128.0, 128.1, 128.2, 128.3, 134.7, 135.1, 135.2, 136.4, 153.1, 154.0, 154.1, 155.8, 156.1, 168.5, 172.5, 203.2; MS (FAB) 1053^+ ($M+H^+$); HRMS (FAB) calcd for $C_{53}H_{69}N_2O_{18}S$ ($M+H^+$) 1053.4266; found 1053.4255; IR (KBr) 3428, 3065, 3033, 2977, 2940, 2881, 1753, 1703, 1636, 1587, 1497, 1455, 1381, 1349, 1329, 1254, 1213, 1161, 1113, 1066, 1027 (cm^{-1}).

4.11. Preparation of compound 11

Compound **11** was prepared from **10** by the procedure described for the preparation of compound **6**.

^{1}H NMR ($CDCl_3$) δ 0.91 (3H, t, J = 7.5 Hz), 1.03 (3H, d, J = 6.0 Hz), 1.26 (6H, d, J = 6.3 Hz), 1.37 (3H, d, J = 6.6 Hz), 1.39 (3H, d, J = 6.9 Hz), 1.47 (3H, s), 1.63 (3H, s), 1.20–1.77 (5H, m), 1.97 (1H, m), 2.30 (6H, s), 2.52 (2H, m), 2.70 (3H, s), 3.01 (1H, quintet, J = 7.8 Hz), 3.22 (1H, dd, J = 7.2 and 9.9 Hz), 3.55 (2H, m), 3.81 (1H, q, J = 6.9 Hz), 4.19 (1H, d, J = 8.1 Hz), 4.31 (1H, d, J = 7.2 Hz), 5.18 (1H, s), 5.58 (1H, dd, J = 2.4 and 10.5 Hz), 9.14 (1H, brs); ^{13}C NMR ($CDCl_3$) δ 10.0, 14.3, 14.5, 15.7, 16.7, 18.7, 19.6, 21.1, 22.0, 25.5, 28.4, 33.2, 38.4, 40.1, 47.7, 49.7, 50.9, 65.8, 69.2, 70.3, 75.1, 78.5, 79.4, 90.4, 91.2, 103.6, 164.0, 168.6, 203.4; MS (FAB) 665^+ ($M+H^+$); HRMS

(FAB) calcd for $C_{30}H_{53}N_2O_{12}S$ ($M+H^+$): 665.3319; found 665.3313; IR (KBr) 3419, 2978, 2939, 2880, 2788, 1755, 1717, 1636, 1457, 1380, 1323, 1277, 1254, 1213, 1161, 1107, 1080, 1052, 1033 (cm^{-1}).

4.12. Preparation of compound 12

Compound **12** was prepared from **7** by the procedure described for the preparation of compound **6**.

^1H NMR ($CDCl_3$) δ 0.86 (3H, t, $J = 7.5$ Hz), 1.00 (3H, d, $J = 6.9$ Hz), 1.15 (3H, d, $J = 6.9$ Hz), 1.23 (3H, s), 1.25 (3H, d, $J = 6.0$ Hz), 1.30 (3H, d, $J = 7.8$ Hz), 1.32 (3H, d, $J = 6.6$ Hz), 1.43 (3H, s), 1.20–1.72 (5H, m), 1.98 (1H, m), 2.29 (6H, s), 2.55 (2H, m), 2.76 (3H, s), 3.12 (1H, quintet, $J = 7.5$ Hz), 3.22 (1H, dd, $J = 7.5$ and 10.2 Hz), 3.27 (1H, brs), 3.57 (1H, m), 3.75 (1H, m), 3.86 (1H, q, $J = 6.9$ Hz), 3.91 (1H, d, $J = 1.5$ Hz), 4.31 (1H, d, $J = 7.2$ Hz), 4.32 (1H, d, $J = 6.3$ Hz), 5.17 (1H, dd, $J = 2.1$ and 10.8 Hz); ^{13}C NMR ($CDCl_3$) δ 10.6, 14.4, 14.5, 14.7, 16.1, 18.4, 19.5, 21.1, 25.3, 28.5, 32.7, 37.9, 40.1, 46.6, 49.9, 50.8, 65.6, 69.2, 70.1, 70.3, 73.7, 77.7, 77.8, 78.2, 103.3, 169.1, 169.5, 204.9; MS (FAB) 603^+ ($M+H^+$); HRMS (FAB) calcd for $C_{30}H_{55}N_2O_{10}$ ($M+H^+$): 603.3857; found 603.3866; IR (KBr) 3425, 2974, 2938, 2877, 2788, 1746, 1715, 1640, 1456, 1404, 1378, 1354, 1336, 1303, 1282, 1253, 1170, 1110, 1075, 1051, 1035 (cm^{-1}).

4.13. Preparation of compound 13

To a solution of compound **3** (750 mg, 0.83 mmol) in toluene (15 mL) was added 2,2-dimethoxypropane (10 mL) and *p*-$TsOHH_2O$ (157 mg, 0.83 mmol) at room temperature, and this was refluxed for 3 days. The reaction mixture was cooled to room temperature and then poured into saturated aqueous $NaHCO_3$ and AcOEt (30 mL). The organic layer was separated and the aqueous layer was extracted with AcOEt (30 mL) again, then the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silicagel (toluene/AcOEt = 8:1–4:1) to give 671 mg of compound **13** as a colorless foam (86%).

^1H NMR ($CDCl_3$) δ 2.75 (3H, two s), 2.83, 2.87 (3H, two s); ^{13}C NMR ($CDCl_3$) δ 101.8, 14.1, 14.3, 15.4, 15.9, 16.9, 19.9, 20.1, 20.5, 22.9, 25.1, 27.8, 28.9, 29.3, 35.6, 36.1, 38.0, 47.8, 49.8, 51.9, 54.6, 67.0, 67.1, 68.5, 69.1, 69.3, 74.0, 74.4, 74.5, 78.8, 81.2, 85.0, 87.4, 101.3, 107.6, 116.4, 127.3, 127.4, 127.5, 127.6, 127.7, 128.0, 128.1, 128.2, 128.3, 134.6, 135.2, 135.3, 136.4, 154.0, 154.2, 155.8, 156.2, 165.8, 169.0, 203.1; MS (FAB) 937^+ ($M+H^+$); HRMS (FAB) calcd for $C_{51}H_{73}N_2O_{14}$ ($M+H^+$): 937.5062; found 937.5078; IR (KBr) 3416, 3065, 2977, 2938, 2878, 1750, 1705, 1497, 1455, 1406, 1379, 1330, 1293, 1252, 1166, 1118, 1066 (cm^{-1}).

4.14. Preparation of compound 14

Compound **14** was prepared from **13** by the procedure described for the preparation of compound **5**.

^1H NMR ($CDCl_3$) δ 2.61 (3H, two s), 2.82, 2.85 (3H, two s); ^{13}C NMR ($CDCl_3$) δ 10.9, 14.2, 14.3, 15.9, 16.9, 17.0, 18.4, 19.7, 19.8, 20.4, 22.9, 25.4, 28.1, 28.8, 34.4, 35.7, 36.1, 38.8, 47.5, 47.6, 49.1, 51.3, 54.6, 67.0, 67.1, 68.4, 69.1, 69.3, 74.6, 78.0, 80.1, 83.6, 83.9, 100.8, 108.0, 127.3, 127.4, 127.6, 127.7, 128.0, 128.1, 128.2, 128.3, 128.4, 135.3, 135.4, 136.4, 154.1, 154.2, 155.8, 156.1, 165.4, 168.9, 203.6, 203.8; MS (FAB) 897^+ ($M+H^+$); HRMS (FAB) calcd for $C_{48}H_{69}N_2O_{14}$ ($M+H^+$): 897.4749; found 897.4733; IR (KBr) 3413, 3090, 3065, 3032, 2979, 2938, 2878, 1751, 1706, 1497, 1455, 1405, 1379, 1350, 1330, 1292, 1249, 1214, 1166, 1117, 1066 (cm^{-1}).

4.15. Preparation of compound 15

Compound **15** was prepared from **14** by the procedure described for the preparation of compound **6**.

^1H NMR ($CDCl_3$) δ 0.88 (3H, t, $J = 7.5$ Hz), 1.08 (3H, d, $J = 6.6$ Hz), 1.23 (3H, d, $J = 5.4$ Hz), 1.25 (3H, d, $J = 7.5$ Hz), 1.35 (3H, d, $J = 4.8$ Hz), 1.39 (3H, d, $J = 6.3$ Hz), 1.41 (3H, s), 1.46 (3H, s), 1.20–1.78 (5H, m), 1.97 (1H, m), 2.29 (6H, s), 2.49 (2H, m), 2.67 (3H, s), 3.09 (1H, m), 3.21 (1H, dd, $J = 7.5$ and 10.2 Hz), 3.53 (2H, m), 3.74 (1H, q, $J = 6.9$ Hz), 4.03 (1H, d, $J = 9.3$ Hz), 4.18 (1H, d, $J = 1.8$ Hz), 4.32 (1H, d, $J = 7.2$ Hz), 5.04 (1H, dd, $J = 3.0$ and 9.3 Hz), 9.2–10.0 (1H, brs); ^{13}C NMR ($CDCl_3$) δ 10.9, 14.7, 16.0, 17.0, 17.9, 19.7, 20.0, 21.0, 23.0, 25.8, 28.2, 28.3, 28.8, 39.0, 40.2, 48.2, 49.3, 51.6, 65.7, 69.2, 70.2, 78.2, 80.1, 81.1, 83.3, 84.1, 103.9, 107.9, 165.7, 169.1, 204.1; MS (FAB) 643^+ ($M+H^+$); HRMS (FAB) calcd for $C_{33}H_{59}N_2O_{10}$ ($M+H^+$): 643.4170; found 643.4163; IR (KBr) 3424, 2978, 2938, 2878, 2838, 2787, 1749, 1714, 1637, 1456, 1378, 1324, 1245, 1213, 1165, 1108, 1076, 1050 (cm^{-1}).

4.16. Preparation of compound 16

To a solution of compound **1** (4 g, 3.78 mmol) in THF (60 mL) was added 1,1′-carbonyldiimidazole (919 mg, 5.67 mmol) and K_2CO_3 (1.04 g, 7.56 mmol) at room temperature, and the reaction mixture was stirred at the same temperature for 2 h. The precipitate was filtered and the filtrate was poured into H_2O and AcOEt (50 mL). The organic layer was separated and the aqueous layer was extracted with AcOEt (50 mL) again and combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was dissolved in THF (60 mL) and the solution was cooled with an ice-water bath. To this solution was added benzyl alcohol (584 μL, 5.67 mmol) and NaH (197 mg, 4.91 mmol), and the reaction mixture was stirred at room temperature for 0.5 h. The reaction mixture was poured into saturated aqueous NH_4Cl and AcOEt (100 mL). The organic layer was separated and the aqueous layer was extracted with AcOEt (100 mL) again, and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silicagel (toluene/AcOEt = 10:1–8:1) to give 2.8 g of compound **16** as a colorless foam (66%).

^{1}H NMR ($CDCl_3$) δ 2.79, 2.83 (3H, two s), 3.03, 3.04 (3H, two s), 3.26, 3.38 (3H, two s); ^{13}C NMR ($CDCl_3$) δ 8.61, 8.69, 10.6, 15.2, 15.9, 16.2, 18.1, 18.2, 18.5, 19.8, 20.8, 20.9, 21.0, 21.2, 26.2, 28.7, 32.8, 35.0, 35.8, 36.3, 36.7, 38.2, 44.6, 48.8, 49.3, 50.7, 54.6, 62.8, 66.8, 66.9, 67.2, 69.2, 69.5, 69.6, 69.7, 70.0, 72.2, 72.3, 73.8, 74.4, 75.0, 75.2, 76.6, 76.7, 77.1, 77.2, 78.2, 79.5, 82.6, 95.2, 95.3, 98.8, 117.2, 127.3, 127.4, 127.6, 127.8, 127.9, 128.1, 128.2, 128.3, 128.4, 134.0, 134.9, 135.0, 135.1, 135.2, 136.3, 135.5, 154.4, 154.5, 155.2, 155.3, 155.9, 156.3, 169.5, 175.0; MS (FAB) 1191^+ ($M+H^+$); HRMS (FAB) calcd for $C_{64}H_{91}N_2O_{19}$ ($M+H^+$): 1191.6216; found 1191.6224; IR (KBr) 3516, 3428, 3066, 3033, 2978, 2940, 2831, 1750, 1705, 1628, 1587, 1497, 1456, 1405, 1382, 1337, 1293, 1254, 1170, 1116, 1073, 1046, 1010 (cm^{-1}).

4.17. Preparation of compound 17

To a solution of compound **16** (1 g, 0.84 mmol) in DMF (15 mL) under cooling with an ice-water bath was added chloroiodomethane (1.53 mL, 21 mmol) and NaH (134 mg, 3.36 mmol) and the reaction mixture was stirred at room temperature for 2 h. Next, the reaction mixture was poured into saturated aqueous NH_4Cl and AcOEt (30 mL). The organic layer was separated and the aqueous layer was extracted with AcOEt (30 mL) again, and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silicagel (toluene/AcOEt = 8:1–4:1) to give 525 mg of compound **17** as a colorless foam (52%).

^{1}H NMR ($CDCl_3$) δ 2.80, 2.83 (3H, two s), 2.94, 3.38 (3H, two s), 3.02, 3.03 (3H, two s); ^{13}C NMR ($CDCl_3$) δ 8.51, 8.60, 10.5, 14.3, 15.6, 16.4, 18.1, 18.2, 19.1, 19.8, 20.8, 20.9, 21.0, 21.8, 26.0, 28.7, 33.7, 34.9, 35.8, 36.3, 36.6, 38.4, 44.8, 48.8, 49.3, 50.2, 54.6, 62.7, 66.8, 66.9, 67.2, 69.2, 69.4, 69.6, 69.7, 72.2, 72.3, 74.1, 75.0, 75.1, 75.2, 76.7, 78.3, 79.5, 80.6, 82.6, 83.4, 95.0, 95.1, 95.2, 98.8, 116.2, 127.3, 127.4, 127.6, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 134.6, 134.9, 135.0, 135.1, 135.3, 136.3, 136.5, 154.3, 154.4, 155.2, 155.3, 155.9, 156.3, 167.4, 175.3; MS (FAB) 1203^+ ($M+H^+$); HRMS (FAB) calcd for $C_{65}H_{91}N_2O_{19}$ ($M+H^+$): 1203.6216; found 1203.6205; IR (KBr) 3440, 3066, 3033, 2975, 2940, 2881, 2832, 1750, 1630, 1587, 1497, 1455, 1382, 1334, 1294, 1255, 1169, 1119, 1098, 1075, 1010 (cm^{-1}).

4.18. Preparation of compound 18

To a solution of **17** (525 mg, 0.436 mmol) in MeOH (11 mL) was added concentrated HCl (12 N, 0.11 mL) under cooling with an ice-water bath, and the mixture was stirred at room temperature for 15 min. The reaction mixture was stirred at 50 °C for 1 h and cooled to room temperature. The reaction mixture was poured into saturated aqueous $NaHCO_3$ and AcOEt (20 mL). The organic layer was separated and the aqueous layer was extracted with AcOEt (20 mL) again, then the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silicagel (toluene/AcOEt = 8:1–3:1) to give 395 mg of compound **18** as a colorless foam (98%).

^{1}H NMR ($CDCl_3$) δ 2.81, 2.85 (3H, two s), 2.94 (3H, s); ^{13}C NMR ($CDCl_3$) δ 7.34, 10.3, 14.3, 15.1, 16.5, 19.1, 19.2, 20.7, 21.8, 25.9, 28.7, 33.5, 35.5, 35.7, 36.2, 36.9, 44.1, 49.4, 54.7, 66.9, 67.1, 68.1, 69.3, 69.5, 74.0, 74.5, 75.3, 75.4, 77.7, 77.9, 80.7, 81.2, 83.3, 95.0, 99.1, 99.2, 116.1, 127.4, 127.5, 127.6, 128.1, 128.2, 128.3, 134.7, 135.0, 135.1, 136.4, 154.1, 154.3, 155.8, 156.2, 167.3, 174.4, 174.5; MS (FAB) 911^+ ($M+H^+$); HRMS (FAB) calcd for $C_{49}H_{71}N_2O_{14}$ ($M+H^+$): 911.4905; found 911.4912; IR (KBr) 3495, 3066, 3033, 2973, 2938, 2878, 2767, 1752, 1735, 1705, 1645, 1587, 1497, 1455, 1407, 1381, 1331, 1293, 1255, 1163, 1120, 1098, 1078, 1004 (cm^{-1}).

4.19. Preparation of compound 19

Compound **19** was prepared from **18** by the procedure described for the preparation of compound **3**.

^{1}H NMR ($CDCl_3$) δ 2.72 (3H, s), 2.81, 2.85 (3H, two s); ^{13}C NMR ($CDCl_3$) δ 10.6, 13.9, 14.3, 15.3, 15.8, 19.1, 19.6, 20.6, 22.3, 26.6, 28.8, 34.6, 35.7, 36.1, 37.7, 46.7, 49.4, 51.1, 54.7, 67.0, 67.1, 68.5, 69.2, 69.4, 74.1, 74.5, 76.5, 78.0, 78.4, 80.1, 83.3, 94.6, 100.5, 116.4, 127.3, 127.4, 127.6, 127.7, 127.9, 128.1, 128.2, 128.3, 134.5, 135.2, 135.3, 136.4, 154.1, 154.2, 155.8, 156.1, 166.4, 168.8, 203.9, 204.2; MS (FAB) 909^+ ($M+H^+$); HRMS (FAB) calcd for $C_{49}H_{69}N_2O_{14}$ ($M+H^+$): 909.4749; found 909.4760; IR (KBr) 3423, 3066, 3033, 2974, 2938, 2877, 2766, 1750, 1704, 1628, 1578, 1497, 1455, 1406, 1381, 1330, 1293, 1254, 1166, 1117, 1098, 1068, 1027, 1005 (cm^{-1}).

4.20. Preparation of compound 20

Compound **20** was prepared from **19** by the procedure described for the preparation of compound **5**.

^{1}H NMR ($CDCl_3$) δ 2.67 (3H, s), 2.81, 2.85 (3H, two s); ^{13}C NMR ($CDCl_3$) δ 10.5, 13.8, 14.1, 15.0, 15.1, 16.4, 18.8, 19.4, 20.6, 22.2, 25.2, 28.8, 33.6, 35.7, 36.2, 37.8, 46.5, 46.6, 49.4, 51.0, 54.8, 67.0, 67.1, 68.5, 69.2, 69.4, 74.6, 76.1, 76.2, 77.7, 78.0, 80.7, 83.0, 94.9, 100.4, 127.4, 127.5, 127.6, 127.7, 128.1, 128.2, 128.3, 135.2, 135.3, 136.5, 154.2, 154.3, 155.9, 156.2, 165.8, 165.9, 168.9, 204.1, 204.4; MS (FAB) 869^+ ($M+H^+$); HRMS (FAB) calcd for $C_{46}H_{65}N_2O_{14}$ ($M+H^+$): 869.4436; found 869.4452; IR (KBr) 3416, 3090, 3065, 3033, 2973, 2938, 2877, 1750, 1705, 1497, 1455, 1381, 1350, 1329, 1293, 1253, 1166, 1116, 1098, 1068, 1006 (cm^{-1}).

4.21. Preparation of compound 21

Compound **21** was prepared from **20** by the procedure described for the preparation of compound **6**.

^{1}H NMR ($CDCl_3$) δ 0.87 (3H, t, J = 7.5 Hz), 1.00 (3H, d, J = 6.9 Hz), 1.16 (3H, d, J = 6.9 Hz), 1.25 (3H, d, J = 6.3 Hz), 1.28 (3H, d, J = 7.5 Hz), 1.34 (3H, d,

J = 6.9 Hz), 1.41 (3H, s), 1.44 (3H, s), 1.20–1.71 (5H, m), 1.85 (1H, m), 2.30 (6H, s), 2.47 (2H, m), 2.73 (3H, s), 3.11 (1H, quintet, J = 7.5 Hz), 3.22 (1H, dd, J = 7.5 and 10.2 Hz), 3.56 (1H, m), 3.73 (1H, m), 3.83 (1H, q, J = 6.6 Hz), 4.21 (1H, d, J = 7.8 Hz), 4.32 (1H, d, J = 7.5 Hz), 4.37 (1H, s), 4.98 (1H, s), 5.05 (1H, dd, J = 2.4 and 10.2 Hz), 5.16 (1H, s); ^{13}C NMR ($CDCl_3$) δ 10.5, 14.1, 14.3, 15.4, 16.3, 18.8, 19.6, 21.1, 22.1, 25.4, 28.4, 33.6, 38.2, 40.2, 47.3, 49.5, 51.1, 65.7, 69.2, 70.2, 76.0, 77.8, 79.2, 80.7, 83.1, 94.8, 103.4, 166.2, 168.9, 204.3; MS (FAB) 615^+ ($M+H^+$); HRMS (FAB) calcd for $C_{31}H_{55}N_2O_{10}$ ($M+H^+$): 615.3857; found 615.3837; IR (KBr) 3423, 2972, 2937, 2877, 2786, 1750, 1716, 1636, 1456, 1379, 1322, 1306, 1277, 1255, 1166, 1141, 1098, 1076, 1051, 1009 (cm^{-1}).

4.22. Preparation of compound 23

Compound **23** was prepared from **22**[6] by the procedure described for the preparation of compound **16**.

^{1}H NMR ($CDCl_3$) δ 2.79, 2.83 (3H, two s), 2.95, 2.96 (3H, two s), 3.26, 3.38 (3H, two s); ^{13}C NMR ($CDCl_3$) δ 8.62, 8.70, 10.5, 15.2, 15.9, 16.2, 18.1, 18.2, 18.5, 19.8, 20.8, 20.9, 21.0, 21.2, 26.4, 28.7, 33.0, 35.0, 35.8, 36.3, 36.7, 38.1, 44.6, 48.8, 49.3, 50.5, 54.6, 62.8, 66.8, 66.9, 67.2, 69.2, 69.4, 69.6, 69.7, 69.9, 72.2, 72.3, 72.5, 73.8, 75.0, 75.2, 76.6, 76.7, 77.0, 77.1, 78.2, 79.5, 82.5, 95.2, 95.3, 98.7, 126.3, 127.3, 127.4, 127.6, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 129.0, 129.3, 132.9, 134.9, 135.0, 135.1, 135.2, 135.5, 136.3, 136.5, 154.1, 154.4, 155.2, 155.3, 155.9, 156.3, 170.7, 175.0; MS (FAB) 1275^+ ($M+H^+$); HRMS (FAB) calcd for $C_{68}H_{92}ClN_2O_{19}$ ($M+H^+$): 1275.5983; found 1275.6001; IR (KBr) 3436, 3065, 3033, 2976, 2939, 2884, 2831, 1750, 1705, 1627, 1574, 1497, 1455, 1404, 1381, 1336, 1293, 1255, 1170, 1116, 1072, 1010 (cm^{-1}).

4.23. Preparation of compound 24

To a solution of **23** (779 mg, 0.61 mmol) in THF (15 mL) was added CS_2 (370 μL, 6.1 mmol) and NaH (100 mg, 2.4 mmol), and the mixture was stirred at 60 °C for 30 min. The reaction mixture was cooled with an ice-water bath, and CS_2 (185 μL, 3.0 mmol) and MeI (380 μL, 6.1 mmol) were added to the mixture. The reaction mixture was sealed and stirred at 60 °C for 2 h. The reaction mixture was cooled with an ice-water bath, CS_2 (185 μL, 3.0 mmol) and MeI (380 μL, 6.1 mmol) were added again to the mixture. This reaction mixture was sealed and stirred at 60 °C for an additional 2 h. The reaction mixture was then cooled to room temperature and poured into saturated aqueous NH_4Cl and AcOEt (30 mL). The organic layer was separated and the aqueous layer was extracted with AcOEt (30 mL) again, and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silicagel (toluene/AcOEt = 15:1–12:1) to give 579 mg of compound **24** as a pale yellow foam (72%).

^{1}H NMR ($CDCl_3$) δ 2.71, 2.78 (3H, two s), 2.78, 2.82 (3H, two s), 2.92, 3.36 (3H, two s); ^{13}C NMR ($CDCl_3$) δ 8.45, 8.53, 10.2, 12.5, 15.5, 16.0, 18.0, 18.1, 18.8, 19.8, 20.9, 21.0, 22.2, 25.9, 28.3, 33.4, 34.9, 35.8, 36.3, 36.6, 38.9, 44.8, 48.8, 49.3, 50.0, 54.5, 62.8, 66.9, 67.2, 69.2, 69.3, 69.6, 69.7, 72.2, 72.6, 74.5, 75.0, 75.2, 76.2, 78.5, 79.2, 82.0, 87.8, 89.4, 95.1, 98.9, 126.3, 127.3, 127.4, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 131.2, 133.4, 134.8, 135.0, 135.2, 135.3, 135.6, 136.3, 136.5, 154.3, 155.2, 155.9, 156.3, 164.8, 175.4, 191.0; MS (FAB) 1317^+ ($M+H^+$); HRMS (FAB) calcd for $C_{69}H_{90}ClN_2O_{19}S$ ($M+H^+$): 1317.5547; found 1317.5564; IR (KBr) 3853, 3674, 3648, 3434, 3065, 3032, 2976, 2940, 2884, 2832, 1748, 1704, 1635, 1574, 1558, 1540, 1497, 1455, 1382, 1355, 1330, 1296, 1254, 1166, 1126, 1071, 1047, 1011 (cm^{-1}).

4.24. Preparation of compound 25

Compound **24** (500 mg, 0.38 mmol) was dissolved in CH_2Cl_2 (20 mL), and diethylaminosulfur trifluoride (DAST) (251 μL, 1.9 mmol) was added to this solution under N_2 atmosphere. This reaction mixture was stirred at room temperature for 3 days under the same atmosphere. The mixture was cooled with an ice-water bath and saturated aqueous $NaHCO_3$ (20 mL) was carefully added. This mixture was poured into AcOEt (30 mL) and H_2O. The organic layer was separated and the aqueous layer was extracted with AcOEt (30 mL) again, and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silicagel (toluene/AcOEt = 12:1–8:1) to give 315 mg of compound **25** as a pale yellow foam (63%).

^{1}H NMR ($CDCl_3$) δ 2.71, 2.78 (3H, two s), 2.82, 2.84 (3H, two s), 2.93, 3.36 (3H, two s); ^{13}C NMR ($CDCl_3$) δ 8.44, 8.54, 10.2, 13.5, 15.6, 16.8, 18.0, 18.1, 19.1, 19.8, 20.8, 20.9, 21.0, 21.9, 26.0, 28.8, 33.1, 34.9, 35.8, 36.3, 36.7, 38.8, 44.9, 48.8, 49.3, 50.0, 54.5, 62.7, 66.9, 67.0, 67.2, 69.1, 69.3, 69.6, 69.7, 72.2, 72.3, 72.4, 75.0, 75.1, 75.2, 75.5, 76.3, 78.4, 79.3, 82.5, 85.6, 86.9, 95.0, 95.1, 98.9, 126.2, 127.3, 127.4, 127.6, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.7, 129.6 (t, J_{CF} = 239 Hz), 130.4, 133.1, 134.9, 135.0, 135.2, 135.3, 136.0, 136.3, 136.5, 154.3, 154.4, 155.1, 155.2, 155.9, 156.2, 166.4, 175.5; MS (FAB) 1323^+ ($M+H^+$); HRMS (FAB) calcd for $C_{69}H_{90}ClF_2N_2O_{19}$ ($M+H^+$): 1323.5749; found 1323.5812; IR (KBr) 3444, 3066, 3033, 2977, 2886, 2833, 1748, 1705, 1631, 1574, 1497, 1455, 1382, 1334, 1254, 1218, 1167, 1115, 1079, 1057, 1047, 1011 (cm^{-1}).

4.25. Preparation of compound 26

To a solution of **25** (300 mg, 0.226 mmol) in MeOH (6 mL) was added concentrated HCl (12 N, 57 μL) under cooling with an ice-water bath, and the mixture was stirred at room temperature for 15 min. The reaction mixture was stirred at 50 °C for 1.5 h and cooled to room temperature. The reaction mixture was poured into saturated aqueous $NaHCO_3$, and extracted with AcOEt (20 mL). The organic layer was separated and the aqueous layer was extracted with AcOEt (20 mL)

again, and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silicagel (*n*-hexane/AcOEt = 8:1–3:2) to give 205 mg of compound **26** as a colorless foam (88%).

^{1}H NMR ($CDCl_3$) δ 2.68 (3H, s), 2.81, 2.85 (3H, two s); ^{13}C NMR ($CDCl_3$) δ 7.25, 10.1, 13.4, 15.1, 16.9, 17.0, 19.1, 19.2, 20.7, 22.0, 25.9, 28.7, 32.8, 35.6, 35.7, 36.2, 37.0, 44.2, 49.1, 54.7, 67.0, 67.1, 68.2, 69.3, 69.5, 72.3, 74.5, 75.6, 77.7, 81.2, 81.3, 85.9, 86.8, 99.2, 99.3, 126.2, 127.4, 127.5, 127.6, 127.7, 128.0, 128.2, 128.3, 128.4, 128.7, 129.6 (t, J_{CF} = 239 Hz), 130.7, 132.8, 133.2, 135.0, 135.1, 136.0, 136.4, 136.5, 154.1, 154.2, 155.9, 156.2, 166.1, 174.4, 174.5; MS (FAB) 1031^+ ($M+H^+$); HRMS (FAB) calcd for $C_{53}H_{70}ClF_2N_2O_{14}$ ($M+H^+$): 1031.4484; found 1031.4486; IR (KBr) 3461, 3066, 3033, 2977, 2938, 2881, 1742, 1704, 1635, 1574, 1497, 1455, 1406, 1382, 1362, 1332, 1291, 1254, 1217, 1166, 1115, 1079, 1058, 1004 (cm^{-1}).

4.26. Preparation of compound 27

Compound **27** was prepared from **26** by the procedure described for the preparation of compound **3**.

^{1}H NMR ($CDCl_3$) δ 2.50 (3H, s), 2.81, 2.85 (3H, two s); ^{13}C NMR ($CDCl_3$) δ 10.4, 13.6, 14.0, 16.0, 19.1, 19.6, 20.6, 22.4, 26.7, 28.8, 35.7, 36.1, 38.0, 47.2, 49.2, 51.2, 54.8, 67.0, 67.1, 68.6, 69.2, 69.4, 72.6, 74.6, 78.2, 85.1, 86.9, 100.6, 126.2, 127.3, 127.5, 127.6, 127.7, 127.9, 128.0, 128.2, 128.3, 128.6, 128.8, 129.3 (t, J_{CF} = 239 Hz), 130.9, 133.4, 135.1, 135.2, 135.8, 136.4, 154.1, 154.2, 155.8, 156.2, 165.2, 168.6, 203.4 203.5; MS (FAB) 1029^+ ($M+H^+$); HRMS (FAB) calcd for $C_{53}H_{68}ClF_2N_2O_{14}$ ($M+H^+$): 1029.4327; found 1029.4344; IR (KBr) 3423, 3066, 3033, 2977, 2938, 2880, 1752, 1704, 1497, 1455, 1382, 1329, 1292, 1254, 1221, 1166, 1113, 1067 (cm^{-1}).

4.27. Preparation of compound 28

Compound **27** (182 mg, 0.18 mmol) was diluted with EtOH (10 mL) and 0.2 M acetate buffer (2 mL, pH 4.4). To this solution was added 20% $Pd(OH)_2$/C (57 mg, 0.1 mmol) and stirred at room temperature under H_2 atmosphere for 6 h. After confirming the disappearance of compound **27** by TLC, 37% aqueous HCHO (1.3 mL) was added to the reaction mixture, and stirred at room temperature under H_2 atmosphere for an additional 2.5 h. The mixture was filtered and concentrated. After being diluted with water, the mixture was basified with 5% aqueous $NaHCO_3$ and then extracted with AcOEt. The resultant residue was purified by column chromatography on silicagel ($CHCl_3$/MeOH = 80:1–20:1) to give 84 mg of compound **28** as a colorless foam (73%).

^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, J = 7.2 Hz), 1.00 (3H, d, J = 6.9 Hz), 1.25 (3H, d, J = 6.0 Hz), 1.25 (3H, d, J = 7.8 Hz), 1.26 (3H, d, J = 6.0 Hz), 1.36 (3H, d, J = 6.9 Hz), 1.47 (3H, s), 1.49 (3H, s), 1.20–1.70 (5H, m), 1.91 (1H, m), 2.72 (6H, s), 2.48 (2H, m), 2.68 (3H, s), 3.04 (1H, quintet, J = 7.8 Hz), 3.21 (1H, dd, J = 7.2 and 10.2 Hz), 3.54 (2H, m), 3.79 (1H, q, J = 6.6 Hz), 4.18 (1H, d, J = 8.1 Hz), 4.30 (1H, d, J = 7.2 Hz), 4.70 (1H, d, J = 3.3 Hz), 5.17 (1H, d, J = 9.0 Hz), 9.26 (1H, brs); ^{13}C NMR ($CDCl_3$) δ 10.3, 13.5, 14.2, 15.8, 16.5, 18.7, 19.5, 21.1, 22.1, 25.3, 28.2, 32.7, 38.4, 40.1, 47.7, 49.6, 51.0, 65.7, 69.3, 70.2, 76.2, 76.3, 78.1, 79.4, 85.9, 86.6, 103.5, 129.336 (t, J_{CF} = 239 Hz), 165.3, 168.6, 203.7; MS (FAB) 651^+ ($M+H^+$); HRMS (FAB) calcd for $C_{31}H_{53}F_2N_2O_{10}$ ($M+H^+$): 651.3668; found 651.3660; IR (KBr) 3425, 2976, 2938, 2880, 2841, 2788, 1752, 1717, 1638, 1457, 1381, 1362, 1324, 1255, 1220, 1167, 1140, 1110, 1049 (cm^{-1}).

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